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Molecular Analysis of Human Pegivirus (GBV-C) Infecting Hemodialysis Patients in Baghdad, Iraq: A Single Center Study

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Abstract

Patients undergoing hemodialysis are at an increased risk of contracting viral infections. The aims of this study were to determine the prevalence of GBV-C infections and to evaluate their clinical importance in hemodialysis patients. This one center cross-section study was carried out for 100 patients attending Al-Kindy center for hemodialysis in Baghdad, Iraq. Serum samples were tested by reverse transcription nested polymerase chain reaction (nested RT-PCR) for GBV-C detection and for liver function evaluation. GBV-C RNA was detected in 35% of hemodialysis patients, 17/48 (35.4%) in HCV positive patients, while 18/52 (34.6%) without HCV. GBV-C infection had no significant association with HCV status among hemodialysis patients, and also no significant association with age, sex and liver enzyme. Patients who have GBV-C have a much longer hemodialysis period than those who do not have GBV-C. Furthermore, phylogenetic analysis showed that ten GBV-C local isolates were related to GBV-C genotype 2; however, two pairs of the local isolates were completely identical (100%), which could be an indicator for nosocomial transmission among hemodialysis unit. In conclusion, GBV-C did not seem to contribute to increasing the level of liver enzyme or the severity of HCV infection in hemodialysis patients, and so mandatory screening for GBV-C is not recommended for hemodialysis patients at this time.

Keyword: HPgV, GBV-C, ALT, AST, hemodialysis, one step nested RT-PCR, phylogenic tree.

1. Introduction

Patients undergoing hemodialysis (HD) therapy are at an increased risk of contracting viral infections. This is due to their underlying impaired cellular immunity, which makes them more susceptible to infection. Furthermore, the HD process necessitates prolonged blood exposure to infectious materials via extracorporeal circulation, which increases the likelihood of nosocomial infection exposure (Bernieh, 2015). Patients undergoing long-term hemodialysis are particularly vulnerable to parenterally transmitted agents, making them an important population for studying the clinical and epidemiological implications of newly discovered agents (Forns, 1999). Hepatotropic or other hepatitis-associated viruses, such as hepatitis B virus (HBV), hepatitis C virus (HCV), torque teno virus (TTV), SEN Virus (SENV), and hepatitis G virus (HGV) are responsible for some of the most common viral infections in hemodialysis patients (Forns, 1999; Ozdarendeli *et al.*, 2005; Abd El-Hady *et al.*, 2006; Abdullah *et al.*, 2012; Abdullah *et al.*, 2014; Khudair *et al.*, 2019).

Human pegivirus (HPgV) or GB virus C (GBV-C), previously known as hepatitis G virus (HGV), was discovered in 1995 and belongs to the Flaviviridae family. It is an enveloped, icosahedral, single-stranded RNA positive-sense virus (9.4 kb) (Ibrahim and Hamdani 2015; Rinonce *et al.*, 2017). GBV-C is classified into seven genotypes with unique distribution depending on the geographic area. Genotypes 1 and 5 are mainly found in Africa, genotype 3 is more prevalent in Asian, in European and North American populations, genotype 4 in the Philippines and Southeast Asia, genotype 6 in China and Japan and 7 is found solely in China. Sometimes, multiple GBV-C genotypes that display a propensity to recombine may infect the same person (Slavov *et al.*, 2019; dos Santos Bezerra *et al.*, 2020). Vertical, parental and sexual transmission of GBV-C have been reported (Sathar *et al.*, 2000). The frequency and occurrence of the GBV-C infection are higher in risk groups that are vulnerable to sexually transmitted or blood-borne infections (Scallan *et al.*, 1998; Christensen *et al.*, 2003).

The clinical significance of GBV-C infection in humans has yet to be determined, and there is a paucity of data in HD patients. The prevalence of GBV-C infection among patients with end-stage renal disease and chronic HD was ranged from 6% to 44% in different countries (Ozdarendeli *et al.*, 2005; Bernieh, 2015). However, none of the studies found that GBV-C infection causes any increase in liver enzymes or hepatic failure, although co-infection with other hepatitis viruses may increase morbidity and mortality rates (Eslamifar *et al.*, 2007;

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** **List of abbreviation**: HPgV= human pegivirus, GBV-C= GB virus type C, ALT= alanine transaminase, AST= aspartate aminotransferase, HD= hemodialysis.

Dadmanesh *et al.*, 2015). The aim of the present study was to determine the prevalence, risk factors and clinical implications of GBV-C infection in hemodialysis patients and to evaluate any possible association with HCV.

2. Materials and Methods

2.1. Study population

The study covered the period from December 2020 to February 2021.This cross-sectional study was conducted on 100 hemodialysis patients in one center at Al-Kindy hospital in Baghdad, Iraq. The participants in this study comprised two groups: those who had HCV antibodies and those who did not.

2.2. Specimen collection

Prior to beginning hemodialysis sessions, 5 ml blood sample was collected from patients as part of the hospital routine work, and sera were isolated from left over blood samples. Blood samples were allowed to clot at room temperature for 20 minutes before being centrifuged at 3,000 rpm for 10 minutes within 2 hours of blood sampling. Aliquots of each sample were made and stored at -20ºC and -70ºC until they were used for biochemical tests (ALT and AST) and reverse-transcriptase nested PCR, respectively. This study was approved by College of Medicine, University Diyala and Ministry of Health (No. 86229 on 8 December 2020).

2.3. RNA Extraction

The serum samples were allowed to thaw at room temperature. For the isolation and purification of RNA, the Viral Nucleic Acid Extraction Kit II (Cat. #VR00, Geneaid, Taiwan) was used. The procedure was carried out following the manufacturer's instructions.

2.4. GBV-C virus RNA Amplification

RNA was converted to cDNA and amplified in the same tube using the SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen, USA). The RT-PCR reaction mixture (25 µL) was prepared using 5 µL of RNA, 1 µl of each of G58 (outer forward primer) and G75 (outer reverse primer) (Table 1), 1 µl of SuperScript™ III RT/Platinum™ Taq Mix, 12.5 µl of the master mix and 4.5 µl nuclease-free water. RNA was transformed to cDNA at 50°C for 30 minutes and then RNA/cDNA hybrid was denatured during the 2-minute incubation at 94°C, followed by 30 cycles of denaturation at 94˚C for 30-second, annealing at 55˚C for 30-second, and extension at 72˚C for 30-second, and final extension for 2-minute at 72˚C. For the second-round reaction, RT-PCR products were further amplified using nested PCR with primers specific for 5-untranslated region (G134 and G131). The reaction mixture (25 µL) was prepared using 5 µL of amplified cDNA from the first PCR run, 1 µl of each of G134 (inner forward primer) and G131 (inner reverse primer), 12.5 µl of the master mix and 5.5 µl nuclease-free water. The cycling conditions for the second-round reaction were performed as following (94˚C for 2-minute, followed by 40 cycles, denaturation at 94˚C for 30-second, annealing at 55˚C for 30-second, and extension at 72˚C for 30-second, and final extension for 2-minute at 72˚C. The PCR products (208 bp) were detected in 3% agarose gel (Figure 1) after electrophoresis for 1 hour at 80 volts.

Table 1. The primers used in nested RT-PCR (Egawa *et al.*, 1996; AbuOdeh *et al.*, 2015).

Primer Name	Product size (bp)	Primer Sequence (5' to 3')
G58 (outer; forward)	242	CAGGGTTGGTAGGTCGTAAATCC
G75 (outer; reverse)	242	CCTATTGGTCAAGAGAGACAT
G134 (inner; forward)	208	GGTCAYCYTGGTAGCCACTATAGG
G131 (inner; reverse)	208	AAGAGAGACATTGWAGGGCGACGT

2.5. Quality control

Samples that previously tested positive were used as positive control samples; they were further confirmed by sequencing, while the negative control consisted of the reagents used to prepare the PCR amplification mixture without GBV-C RNA.

2.6. Sequencing and phylogenetic analysis

Ten PCR products of second-round PCR of GBV-C and G134 (inner; forward) primer were sent for Sanger sequencing by Microgen Corporation (Korea). The results were analyzed using Bio Edit v7.2.5. The phylogenetic relationships between the ten GBV-C samples were reconstructed in the form of a Neighbor-Joining tree by Mega Xv.10.2.5+NCBI and based on the Jukes-Cantor (JC) model. Genotypes of GBV-C were provisionally estimated from the tree based on the clustering with the reference GBV-C isolates from GenBank (GenBank acc. AB013206.1, AF095693.1, GQ227348.1, GQ380413.1, Y18156.1, JX494215.1, AY269959.1, AF075218.1, AF058742.1, AY196904.1, AF078055.1, AJ000584.1, AF172512.1, AB033840.1, AB022539.1, JF832375.1, U86113.1, AF073743.1, AF015876.1). Our isolates were deposited at GenBank under the acc. MW962987.1, MW962988.1, MW962989.1, MW962990.1, MW962991.1, MW962992.1, MW962993.1, MW962994.1, MW962995.1 and MW962996.1.

2.7. Statistical Analysis

All data were analyzed using the statistical package for social sciences (SPSS), version 26. Data were expressed as means ± S.D. The chi-squared test ($\chi 2$) was used to determine the frequency difference, and Student's t-test was used to evaluate the mean differences. ANOVA test was used to compare the means of more than two independent groups. The level of significance in all cases was set at a two-tailed ($p<0.05$).

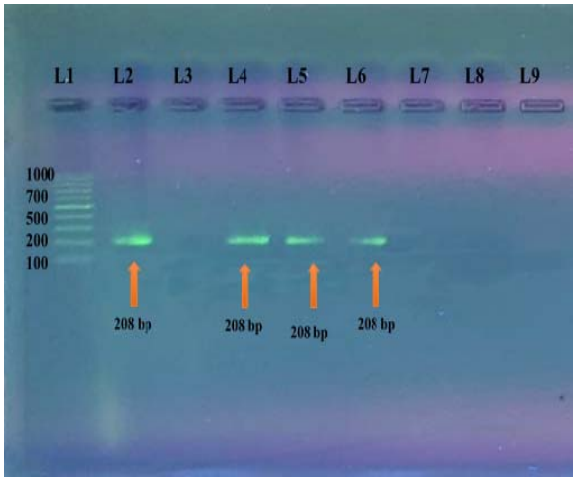

Figure 1. Gel electrophoresis of GBV-C second round PCR product using 3% agarose in TBE buffer, L1 was (100-1000 bp) ladder. L2, L4, L5, and L6 were positive samples (208 bp), while L3, L7, L8, and L9 were negative samples.

3. Result

This study included 100 hemodialysis patients with a mean age of 51.17 years (±15.69 SD). Forty-eight (48) were HCV positive, while 52 were HCV negative, with mean ages of 49.9 years (±15.097 SD) and 51.6 years (±16.635 SD), respectively. Males made up 60% of the population, while females made up 40%. GBV-C was found in 35 out 100 (35%) hemodialysis patients, 17 out 48 (35.4%) HCV positive patients, and 18 out 52 (34.6%) HCV negative patients. There was no significant association (p> 0.05) between GBV-C infection and HCV status among the hemodialysis patients (Table 2). In addition, there was no statistically significant difference (p> 0.05) between GBV-C positive and GBV-C negative individuals in terms of liver enzyme levels (Table 3).

Table 2. Occurrence of GBV-C among hemodialysis patients with respect to HCV status.

GBV-C RNA Status*	HCV status		Total hemodialysis patients (n=100)	Statistic
	HCV Positive (n=48)	HCV Negative (n=52)		
GBV-C Positive	17 (35.4%)	18 (34.6%)	35 (35%)	
GBV-C Negative	31 (64.6%)	34 (65.4%)	65 (65%)	p= *0.933*
Total	48 (100%)	52 (100%)	100 (100%)	

*Using Chi-square test at 0.05 level

Table 3. Serum ALT and AST level (U/L) in relation to GBV-C status in hemodialysis patients (n=100).

Biochemical tests*	GBV-C		Statistic
	Positive (n=35)	Negative (n=65)	
ALT (U/l)**	5.460 ± 5.093	6.605 ± 6.101	p= 0.619
AST (U/l)**	7.111±5.437	8.875 ±7.296	p= 0.213

**Normal values: AST 15-37 U/L, ALT 12-78 U/L

*Using T- test at 0.05 level.

Concerning the demographic data, clinical characteristics and risk factors of participants, there is no significant statistical difference (p>0.05) between the GBV-C positive and negative individuals. However, the duration of hemodialysis is significantly (p<0.05) longer in GBV-C positive patients than in GBV-C negative patients (Table 4).

For GBV-C genotyping, ten GBV-C positive samples were successfully sequenced using the 5'-untranslated region (5'-UTR). The sequences of the current study were compared with GBV-C reference sequences from the GenBank database. The sequences of the local GBV-C isolates were 96-99% similar to that of the reference GBV-C genotype 2 and clustered in a common group with the sequences of GBV-C isolated from Bolivia, United Kingdom, Brazil, Canada, Belgium, Venezuela, France, China, Hong Kong, USA, Singapore, Germany, South Africa, Yamagata, Japan, Colombia, Sweden, Greece and Italy (Table 5)

Nucleotide sequence alignment showed 99% similarity between local isolate sequences in current study with Bolivian isolate GenBank Accession number (AB013206.1). However, these local isolates (GB virus C IRAQ:3, 4, 5, 14, 19, 37, 46, 60, 62 and 76) showed some variations summarized in the table (6).

Phylogenetic trees of GBV-C (5-UTR) detected in hemodialysis patients were constructed using Neighbor joining (NJ) tree based on the Jukes-Cantor (JC) model and evaluated using the interior branch test method with (MEGA version 10.2.5). All local GBV-C (5-UTR) sequences were grouped in the same cluster with 99% similarity (Figure 2). These sequences belong to genotype 2 and were clustered in a common group (Figure 3) with GBV-C sequences from: Bolivia (AB013206.1), United Kingdom (AF095693.1) and Belgium (Y18156.1) with 99%, 98%, 97% similarity, respectively.

Table 4. Comparison of clinical characteristics and risk factors according to GBV-C status in hemodialysis patients (n=100).

Risk factors*		GBV-C		Total	Statistic
		Positive	Negative		
Age (mean±SD)**		50.94±13.70	51.29±16.77	51.17±15.69	p=0.198
Sex	Female	14 (39.6%)	26 (40.4%)	40 (40%)	p=1.000
	Male	21(60.4%)	39 (59.6%)	60 (60%)	
Diabetes Mellitus	Presence	14(40%)	19(29.2%)	33 (33%)	p=0.275
	Absence	21(60%)	46(70.8%)	67 (67%)	
	Total	35(100%)	65(100%)	100(100%)	
Hypertension	Presence	26(74.29%)	52 (80%)	78 (78%)	p=0.629
	Absence	9 (25.71%)	13 (20%)	22 (22%)	
	Total	35 (100%)	65 (100%)	100(100%)	
History of blood transfusion	Presence	30(85.71%)	47 (72.3%)	77 (77%)	p=0.499
	Absence	5 (14.29%)	18 (27.7%)	23 (23%)	
	Total	35 (100%)	65 (100%)	100(100%)	
History of kidney transplantation	Presence	1 (2.85%)	1 (1.56%)	2 (2%)	p=0.653
	Absence	34(97.15%)	64(98.44%)	98 (98%)	
	Total	35 (100%)	65 (100%)	100(100%)	
History of surgery	Presence	21 (60%)	37(56.92%)	58 (58%)	p=0.504
	Absence	14 (40%)	28(43.08%)	42 (42%)	
	Total	35 (100%)	65 (100%)	100(100%)	
History of multiple sexual partners	Presence	30(85.71%)	57(87.69%)	87 (87%)	p=0.738
	Absence	5(14.29%)	8(12.31%)	13 (13%)	
	Total	35 (100%)	65 (100%)	100(100%)	
History of Tattooing	Presence	4 (11.42%)	7 (10.76%)	11 (11%)	p=0.920
	Absence	31(88.58%)	58(89.24%)	89 (89%)	
	Total	35 (100%)	65 (100%)	100(100%)	
Hemodialysis duration	< 1 y	6(17.14%)	29(82.86%)	35 (35%)	p=0.02
	1-2 y	8 (36.36%)	14(63.64%)	22 (22%)	
	2-4 y	9 (60%)	6 (40%)	15 (15%)	
	> 5 y	12 (42.85%)	16 (57.15%)	28 (28%)	
No. of blood transfusions	0-2 time	21(32.3%)	44 (67.7%)	65 (65%)	p=0.71
	2-4 time	10 (41.66%)	14(58.34%)	24 (24%)	
	> 5 time	4 (36.36%)	7 (63.64%)	11 (11%)	

*Using Chi-square test.

**Using T-test at 0.05 level, S.D: Standard deviation

Table 5. The similarity of the local GBV-C sequence with that of isolates from different countries

No.	Accession	Country	Similarity
1	ID: AB013206.1	Bolivia	99%
2	ID: AF095693.1	United Kingdom	98%
3	ID: GQ227348.1	Brazil	98%
4	ID: GQ380413.1	Canada	98%
5	ID: Y18156.1	BELGIUM	97%
6	ID: JX494215.1	Venezuela	97%
7	ID: AY269959.1	France	97%
8	ID: AF075218.1	China	97%
9	ID: AF058742.1	Hong Kong	97%
10	ID: AY196904.1	USA	97%
11	ID: AF078055.1	Singapore	97%
12	ID: AJ000584.1	GERMANY	97%
13	ID: AF172512.1	South Africa	96%
14	ID: AB033840.1	Yamagata	96%
15	ID: AB022539.1	Japan	96%
16	ID: JF832375.1	Colombia	96%
17	ID: U86113.1	Sweden	96%
18	ID: AF073743.1	Greece	96%
19	ID: AF015876.1	Italy	96%

Table 6. Local GBV-C gene 5'UTR point mutation comparing with Bolivian isolate (AB013206.1).

No	Local isolate	Type of substitution	Location	Nucleotide	Similarity
1	1-3 GB virus C IRAQ	Transition	160	C\T	99%
		Transition	198	G\A	
2	2-4 GB virus C IRAQ	Transition	144	A\G	99%
		Transition	198	G\A	
3	3-5 GB virus C IRAQ	Transition	144	A\G	99%
		Transition	198	G\A	
4	4-14 GB virus C IRAQ	Transvertion	72	C\A	98%
		Transition	160	C\T	
		Transvertion	184	T\A	
		Transition	198	G\A	
5	5-19 GB virus C IRAQ	Transition	144	A\G	98%
		Transvertion	152	A\T	
		Transvertion	184	T\A	
		Transition	198	G\A	
6	6-37 GB virus C IRAQ	Transition	49	C\T	99%
		Transition	71	C\T	
		Transition	144	A\G	
7	7-46 GB virus C IRAQ	Transvertion	152	A\T	98%
		Transvertion	184	T\A	
		Transition	198	G\A	
8	8-60 GB virus C IRAQ	Transition	49	C\T	98%
		Transvertion	61	C\A	
		Transition	164	C\T	
		Transition	198	G\A	
9	9-62 GB virus C IRAQ	Transvertion	45	G\C	99%
		Transition	160	C\T	
		Transition	198	G\A	
10	10-76 GB virus C IRAQ	Transition	160	C\T	99%
		Transvertion	184	T\A	
		Transition	198	G\A	

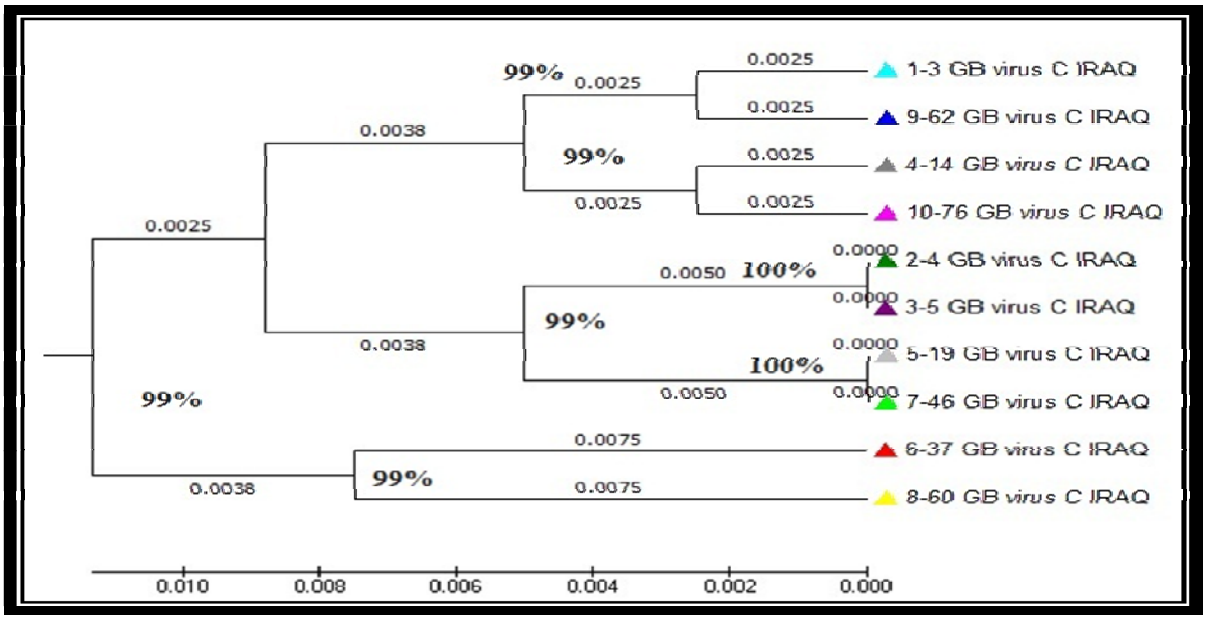

Figure 2. Phylogenetic trees for 5'UTR of 10 local isolates of GBV-C constructed by the neighbor joining method.

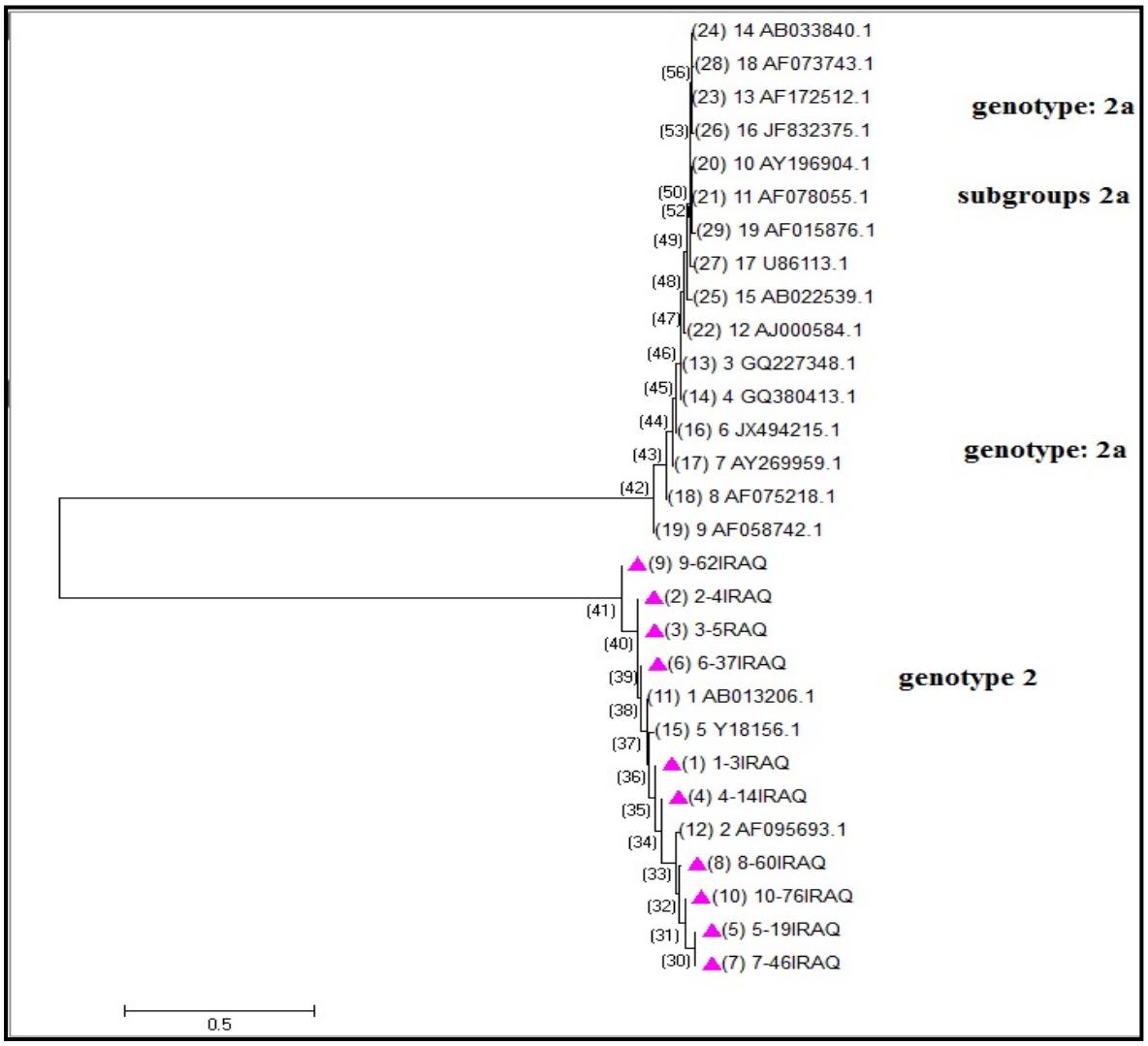

Figure 3. Phylogenetic trees for 5'UTR of 10 local isolates of GBV-C and 19 reference isolates constructed by the neighbor joining method. Local isolates were flagged with pink colored triangle. The scale bar under the tree indicates 0.5 nucleotide substitutions per site.

4. Discussion

In the current study, GBV-C was found in 35 (35%) of hemodialysis patients, 17 (35.4%) of whom were HCV positive and 18 (34.6%) were HCV negative, Table (2). This prevalence is within the range reported in other countries including Indonesia (55%) (Handajani *et al.*, 2000), Germany (50%) (Sanchooli *et al.*, 2018), Turkey (25%) (Akcali *et al.*, 2006), Italy (24%) (De Filippi *et al.*, 2001), Iran (10%) (Sanchooli *et al.*, 2018), and Pakistan (3.6%) (Qureshi *et al.*, 2020). Variable rates of blood transfusion, surgical procedure and adherence to universal precautions may explain these disparities in GBV-C prevalence. The variation in GBV-C prevalence around the world could be attributed to variation in amplified target region and primers used (Sanchooli *et al.* 2018). In Iraq, the rate of GBV-C infection among healthy people was 0%, hemodialysis was 20.3%, and thalassemia was 14.8% (Hasan *et al.*, 2018). Another study found that HGV infection was detected in 25% of blood donors, 30% of chronic hepatitis C and 25% of chronic hepatitis B (Al-Obeidy *et al.,* 2010).

According to Table (3), there was no significant association between the levels of ALT and AST in hemodialysis patients with or without GBV-C infection, indicating that GBV-C infection did not cause more serious liver damage in those patients and that GBV-C did not appear to influence liver enzyme levels. Salama and Selim (2009), on the other hand, showed a significant difference in ALT and AST values between GBV-C positive and negative patients. There have been many debates about the pathogenicity of GBV-C since it was identified; several studies have shown that GBV-C can cause acute, chronic and even fulminant hepatitis (Zhu et al. 2003). Paradoxically, GBV-C infection has been shown to have little significance in causing liver damage in humans (Zhu et al. 2003; Alhetheel and El-Hazmi 2014; Nasidi and Rogo 2017). The current findings support the findings of Alhetheel and El-Hazmi (2014), who stated that there were no significant differences in liver enzyme (ALT and AST) levels among HBV or HCV infected patients who were also infected with GBV-C.

This study found no statistically significant difference in diabetes mellitus (DM), hypertension, history of blood transfusion, history of kidney transplantation, history of surgery, history of multiple sexual partners, history of tattooing, or number of blood transfusions between GBV-C positive and negative individuals ($p>0.05$). Hemodialysis duration is significantly ($p<0.05$) longer in

GBV-C positive individuals (Table 4). In the current study, 40% of GBV-C positive patients had DM and 74.29% had hypertension; however, there was no significant association between infection of GBV-C and DM or hypertension. Other studies reported a lower percentage (13.1-15%) of DM and (21.3%) hypertension (Izumi *et al.*, 2019; Savassi-Ribas *et al.*, 2020). This disparity could be attributed to differences in the study population, their underlying medical condition, age, and sample size. In addition, there was no significant association between GBV-C infection and a history of blood transfusion, despite the fact that 85.71% of hemodialysis patients who were GBV-C positive had a history of blood transfusion, and 73.96% had blood transfusions frequently. This is consistent with the findings of Ibrahim and Hamdani (2015), who found no significant differences in the prevalence of GBV-C RNA among age groups, sex, or frequency of blood transfusion. Also, Salama and Selim (2009) reported that there was no significant association between blood transfusion or products of blood, with or without GBV-C infection.

Kheirabad *et al.* (2016) demonstrated a significant association between the prevalence of viruses, such as HCV and HIV in HD patients and GBV-C. Recently, the following strategies have emerged to reduce the risk of infections following blood transfusion: (1) careful donor selection, (2) regular screening of blood donors for HBV, HCV, HIV, human T-cell viruses, and cytomegalovirus (in high-incidence areas), and (3) virus removal and inactivation, particularly double inactivation of blood products and plasma derivatives, excluding complete blood or RBC components (Ghanbari *et al.*, 2010; Kelishadi *et al*., 2019). The history of kidney transplantation and history of surgery also had no significant differences in prevalence of GBV-C, which is in agreement with Rinonce *et al.* (2017). In contrast, Vogt *et al.* (2006) reported that there was a higher correlation between the infection of GBV-C and the number of operating surgery and blood transfusion. In addition, there was no significant association between GBV-C infection and the history of multiple sexual partners and tattooing, which is in consistence with that reported by Rinonce *et al.* (2017). But the high incidence of GBV-C-RNA in intravenous drug users and their heterosexual partners supports that sexual contact may help virus spread in some groups, in addition, the presence of identical sequences in both husband and wife, suggesting that one person was the source of the transmission, however, it is unknown if the transmission occurred sexually or through other unidentified means (Mphahlele *et al.,* 1998).

Many studies have reported that the duration of hemodialysis was a risk factor for GBV-C infection supporting patient-to-patient transmission (Hasan *et al.*, 2018; Sanchooli *et al.,* 2018), which is consistent with the current study finding. This could be attributed to nosocomial infections as a result of hemodialysis units failure to adhere to strict infection control procedures. Although the frequency of GBV-C was only 35%, the current study may have underestimated the true prevalence of GBV-C because E2 antibodies were not used to assess the history of GBV-C infection, and only active GBV-C infection was measured based on viral RNA amplification (Rinonce *et al.,* 2017). Other studies found no significant difference between the long hemodialysis duration and infection with GBV-C (Hosseini-Moghaddam *et al.,* 2008; Rinonce *et al.,* 2017).

Alignment of local isolates 5'-UTR sequences demonstrated that these sequences were sharing (96-99%) similarity with GBV-C reference sequences from Bolivia, United Kingdom, Brazil, Canada, Belgium, Venezuela, France, China, Hong Kong, USA, Singapore, Germany, South Africa, Yamagata, Japan, Colombia, Sweden, Greece and Italy, as shown in (Table 5). However, the results show that all local isolates are very similar, at least using the 5'-UTR, to the Bolivian reference isolate sequence (GenBank accession number AB013206.1), although some substitution mutations (transition and/or transversion) were seen (Table 6). A significant amount of research on this virus revealed some genomic diversity, particularly when the 5'-UTR region of the gene which was employed for phylogenetic analysis (Ibrahim and Hamdani 2015; Slavov *et al.,* 2019)**.** RNA viruses are known to develop a variety of subtypes (quasi-species) providing advantage for viruses to evade host defense mechanisms. Indeed, the variability of 5′-UTR areas in GBV-C greatly contributes to genotype diversity in this virus (Vitrenko *et al.,* 2017).

There is little variation, at least in the 5'-UTR, amongst local isolates themselves. The ten local strains clustered together in the phylogenetic tree with (99%) similarity. The high similarity between the ten GBV-C local isolates sequences in the current study (Figure 2) might be due to the nature of the 5'-UTR selected for amplification and sequencing; this is a highly conserved region with few genomic variations (Chivero and Stapleton, 2015). In addition, the amplified fragment was relatively short (208 bp), which puts a limitation on phylogenetic information that help distinguish the amplified sequences. The epidemiological profile of studied group (hemodialysis patients) may also have influenced this high similarity between the sequences. The 5'-UTR region of GBV-C was chosen because it is well conserved among isolates and easy to amplify (Da Mota *et al.*, 2019; Rinonce *et al.*, 2017).

All ten GBV-C local isolates in the current study were related to genotype (2), and all our isolates clustered with the Bolivian isolate (AB013206.1), United Kingdom isolate (AF095693.1) and Belgian isolate (Y18156.1) with 99%, 98%, 97%, respectively, as shown in (Figure 3). GBV-C isolates belonged to genotype 2, which is common in North America/Europe. This genotypic profile is similar to that reported in other previous studies in blood donors from Kuwait, Jordan and Emirati (AbuOdeh *et al.,* 2015), and in Iraqi thalassemia patients (Ibrahim and Hamdani, 2015), which also clustered with genotype 2. As a result, it appears that many regions in the Middle East have a similar genetic pattern of GBV-C to North America and Europe, which might be due to reciprocal immigration and traveling between these countries. Therefore, the predominance of genotype 2 in the current study could suggest patient-to-patient transmission owing to the predominance of this genotype in the general population. Interestingly, two pairs were completely identical (100%), particularly in the 5'-UTR sequences (2-4 GB virus C IRAQ and 3-5 GB virus C IRAQ) and (5-19 GB virus C IRAQ and 7-46 GB virus C IRAQ) among local isolates, as shown in figure 2, which could be due to nosocomial

transmission among patients in hemodialysis unit as reported by Rinonce *et al.*, (2017).

In conclusion, GBV-C did not seem to contribute to increases in the level of liver enzyme or the severity of HCV infection in hemodialysis patients. Based on phylogenetic analysis of the 5'-UTR, the current study found that local isolates were closely similar to each other and to similar reference known strains, suggesting that GBV-C infection of genotype 2 is one of the prevalent genotypes in Iraq. The limitation of current study is that only 10 PCR products of positive samples were sequenced; however, it is considered as a preliminary study on GBV-C genotyping that could pave the way for further studies with larger samples size from different regions of Iraq to shed light on the actual GBV-C prevalence of different genotypes in our country.

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Author contributions

As part of his MSc, Hayder A. Kadhim performed all laboratory work, statistical analysis and wrote the draft of this paper. This work was designed, supervised and reviewed by Dr. Arwa M. Al-Shuwaikh and Dr. Ismail I Latif. The final version of this manuscript was read and approved by the authors.

Conflict of Interests

The authors state that they do not have any conflicting interests.

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